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[From the PHARMACEUTICAL JOURNAL for November, 1852].

RESEARCHES ON THE COLOURING PRINCIPLE OF URINE— EXISTENCE OF IRON IN THAT LIQUID.

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THE most obvious character possessed by all extractive matter, whether obtained from the animal fluids or solid tissues, is its colour; and it is much to be regretted that notwithstanding the improved methods of investigation in Physiological Chemistry, the term extractive matter has still to be so frequently employed by practical Chemists to designate those bodies which, whether existing preformed, or chemically produced, possess few or no distinguishing characters, being uncrystallizable, incapable of forming a crystallizable or stoichiometrical compound with other substances, &c., and therefore cannot be separated or exhibited in a pure state. In this class the colouring matter of urine, and it may be several other substances, are involved.

The nature of the pigment which bestows upon urine, and urinary sediments, their colour, has hitherto never been properly understood in consequence, of observers being unable to separate it from the other substances to which it tenaciously adheres, sharing their solubility and insolubility. "Experiments have often been commenced upon this substance, but the difficulties which present themselves in the investigation are so numerous that most experimentalists have soon resigned it, and directed their labours to some more productive department of chemistry. It unfortunately happens that no certain chemical differences can be detected between urines presenting the most striking difference of colour to the eye of the clinical physician."*

In a paper such as this it would be out of place to give details respecting the nature of the substances which have been described as imparting to urine its red or yellow tint; suffice to say that the colouring matter of urine has been called and described as purpurate of ammonia by Prout, purpurine by Bird, uroerythin by Simon, urrhodin by Steller, uroxanthin by Heller,† and more lately as urine pigment by Scherer.‡

None of these observers are agreed as to its nature, but this difference of opinion cannot be wondered at when we consider that none of them ever obtained the pigment in a pure state so as to be able to submit it to exact chemical examination. By the following process I have obtained it pure, and after a careful study of its properties have come to the conclusion that it is a modification of the hæmatin which exists in the blood, and like it containing iron, as I have been able to demonstrate by taking the residue after combustion, dissolving it in hydrochloric acid, and adding the usual tests for that metal, when the characteristic colours are immediately produced. In operating on urine I have not confined myself to that of man alone, but also employed the urine of several of the lower animals, such as the ox, horse, pig, &c., and in all of them I have found iron existing as a normal constituent combined with the colouring matter. This, as will be afterwards seen, is a most important observation.

The method I employ to obtain the colouring principle of urine in a state of purity, consists in evaporating a very large quantity of urine almost to dryness, taking care during the process to remove the chloride of sodium and other salts, which by crystallizing in great abundance in the bottom of the vessel, and on the surface of the liquid, greatly retard evaporation. When the urine has been evaporated till there remains in the vessel a semifluid matter having somewhat the colour and consistence of molasses, it is removed from the

* Lehmann's *Physiological Chemistry*, translated by Dr. Day, vol. i., page 318.

† *Arch. f. Chem. u. Mikrosk.* Bd. 2, S. 161, 173.

‡ *Ann. d. Ch. u. Pharm.* Bd. 57, S. 180, 195.

water-bath, and alcohol is added to extract the colouring matter. The alcohol on becoming saturated is decanted into another vessel, and the residue is treated with fresh quantities till it ceases to yield colouring matter. The alcoholic extract having a dark red colour is heated till it boils, and whilst boiling, slacked lime is added in small quantities at a time till the liquid becomes decolorized, the vessel being well shaken after each addition; an excess of lime is to be avoided, as it renders the after part of the process more difficult. The decolorized liquid is now filtered to obtain the compound of lime and colouring matter which remains on the filter, and the alcohol which passes through carries with it fats, a little colouring matter, &c. This compound, having been well dried, is repeatedly washed with boiling ether, to free it still further from fats, of which there is always a considerable quantity in urine. This treatment is continued till a portion of the washings no longer leaves a fatty stain when evaporated in a watch-glass. After the lime compound has been again dried, it is acted upon by hydrochloric acid, which decomposes it, and sets free the colouring matter, and the treatment with alcohol is repeated to separate it from the lime. After filtration ether is added to the solution in about equal parts, and the mixture is allowed to stand for a few days to insure that as much as possible of the pigment be taken up by the ether; on the addition of water the combination of ether and colouring matter forms a distinct upper layer which is removed without difficulty. The ethereal solution thus obtained has a rich port wine colour, and contains the pigment in a pure state, with the exception, perhaps, of a very slight trace of acid, should the hydrochloric acid have been added in excess. To free it from this it is put into a funnel, the small end of which had been stopped by the finger, and distilled water poured upon it; the water combines with the acid, and sinks to the lower part of the funnel, from which it is withdrawn by partially removing the finger. In the process of washing, care must be taken not to add an excess of water, as it precipitates some of the colouring matter. All that now remains to be done with the ethereal solution is to distil or evaporate the ether, and there is left the pigment, which, on being dried, appears as a brownish-black glistening mass.

The colouring matter of urine thus obtained is insoluble in water, soluble in alcohol and ether, separately or combined, more freely soluble in absolute alcohol. The presence of fat or oil adds greatly to its solubility. On being burned it evolves an odour similar to burning horn, and leaves a slight residue soluble in hydrochloric acid, which is recognized to be a salt of iron by the ferrocyanid. pot., and ferricyanid. pot. giving with this solution the one a pale blue, and the other Prussian blue. The sulpho cyanid. pot. gives a fine red colour. In consequence of an accident I am unable at present to give an ultimate analysis with the exact numbers of the elements contained in it; suffice it to say that it contains carbon, hydrogen, oxygen, nitrogen, and iron. These properties prove the very close resemblance which it bears to blood hæmatin, and justify me in considering it a modified form of that substance, the only difference appearing to be in the quantity of iron which it contains. The colouring principle of the bile and also melanin bear the same close resemblance to the hæmatin of the blood as urine pigment, the amount of iron in bile pigment seems to be less, while in melanin it is greater. I found as much as one and a half per cent. of iron in melanin prepared from a melanotic tumour taken from a horse. A most remarkable character possessed by all of these pigments when pure is that they are insoluble in the stronger acids, as acetic, nitric, hydrochloric, and even sulphuric acid. The colouring matters of plants are very nearly allied to those of animals, as will be seen from researches now in print made by my former teacher, M. Verdeil, who has lately devoted much of his time to the investigation of this interesting subject, and with the most satisfactory results.

Having, as I hope, given a satisfactory answer to the question, "What is the colouring principle of urine?" and given a process by which it may be obtained in a state of purity, I will leave the subject till a future period, when I hope to be able to explain it more minutely.

Paris, 28th Sept., 1852.

